FORSCHUNGEN
AUF DEM
GEBIET DER PFLANZENKRANKHEITEN
(Shokubutsu Byogai Kenkyu)

Herausgegeben von

Prof. Dr. Shigeyasu Akai
Universität zu Kyoto

Heft VII, Nr. 2

Physiological studies on the formation and germination of sporangia of *Phytophthora infestans* (Mont.) DeBary

Masaki Yamamoto and Junichi Tanino

Kyoto, Japan
1961

Forsch. Gebiet Pflanzenkrankh. (Shokubutsu Byogai Kenkyu), Kyoto 7(2): 7~22, 1961

Physiological studies on the formation and germination of sporangia of *Phytophthora infestans* (Mont.) DeBary*

Masaki Yamamoto and Junichi Tanino

1. Introduction

Late blight is known as the most serious disease of potatoes and physiological elucidation of *Phytophthora infestans*, the primary cause of this disease, might be of importance for a study of this disease. Regarding the sporulation, constituted elements of the medium and their concentration, carbon and nitrogen sources, C-N ratio, rare elements and vitamins and the influence of temperature, moisture, light, aeration, hydrogen-ion concentration have to be considered. On the other hand, conidia (zoosporangia) of *Phytophthora infestans* germinates directly by germtube at comparatively higher temperatures (20-23°C) and indirectly by liberating swarmspores at rather lower temperatures (11-13°C). To elucidate the physiological mechanism of these different types of conidial germination, several observations and experiments were carried out.

2. Sporangial formation of *Phytophthora infestans*

Influence of the quantity of nitrogen and carbon sources in media on the sporangial formation, and the sporangial formation on potato leaves were investigated. Components of leaves, especially various types of nitrogen and sugars were considered as a condition of sporangial formation.

1) Influence of carbon and nitrogen sources to the mycelial growth and sporulation of *Phytophthora infestans*

In order to investigate the influence of sugar content and nitrogen sources to the growth of *Phytophthora infestans* and to elucidate the relation to the sporulation, the following experiments were carried out.

Race O of *Phytophthora infestans* was used, and as a culture medium Tochinai and Nakano's solution was selected. Potassium nitrate and glucose were used as nitrogen and carbon sources respectively. The hydrogen-ion concentration was adjusted around pH 6.0 and the medium was solidified by adding 5% of agar. 20ml of these media were taken to Petri dishes and the fungus was inoculated and incubated at 20°C. 3 dishes were used in each plot. At the interval of 5 days, the diameter of the fungus colony and the sporulation degree was measured three times.

a) Glucose content and the mycelial growth and sporulation of *Phytophthora infestans* Sakai[10] in his studies of *P. infestans* stated that among carbohydrates,

* A part of these investigations was supported by a Grant in Aid for Fundamental Scientific Reserches from the Ministry of Education

** Laboratory of Plant Pathology, Shimane Agricultural College, Matsue, Japan

glucose showed the maximum growth of the fungus, galactose stimulated the growth a little, but fructose did not cause the mycelial growth. Sucrose also showed the good mycelial growth. Maltose and lactose gave the same level of growth as galactose, and inuline showed good mycelial growth, but soluble starch did not show any evidence of growth. Enomoto[3] reported that maltose, sucrose and galactose are suitable for the mycelial growth and sporulation of fungus.

Table 1 Glucose content in medium and the mycelial growth and sporulation of *Phytophthora infestans*

Content of glucose mg/20cc	Days elapsed after inoculation 5	10	15	20	25	30	35	40	Spore formation	H-ion concentration of medium (pH)
0	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	-	6.0
10	0.7	0.9	1.1	1.4	1.8	2.0	2.5	3.4	++	5.8
20	0.8	0.9	1.3	1.9	2.2	3.1	3.4	4.3	++	5.8
30	0.7	1.2	1.3	2.6	3.1	4.0	5.1	4.7	++	5.7
40	0.9	1.8	2.3	3.9	4.9	5.4	6.4	6.3	+++	5.6
50	0.8	2.3	2.5	4.9	5.6	5.5	6.2	6.6	+++	5.6
60	0.6	1.0	1.6	2.0	2.2	3.5	4.2	5.1	++	5.6
70	0.6	1.3	2.7	3.3	3.5	4.0	4.5	5.0	+	5.6
80	0.8	1.2	1.7	2.0	2.9	3.5	3.9	4.2	+	5.6
90	0.8	1.2	1.5	2.3	3.3	3.8	3.8	4.2	-	5.9

* Diameter of mycelial mat (cm)

** + means 10, ++ means 11—20, +++ means more than 21 conidia in one microscopic field under 80 magnification

According to Table 1, 40 mg and 50 mg of sugar per 20 cc medium showed vigorous mycelial growth and sporulation. Scanty sporangial formation was recognized in the plots containing more than 80 mg and no formation in the plots where its quantity was higher than 90 mg.

b) Content of potassium nitrate and the mycelial growth and the sporulation of *Phytophthora infestans* According to Table 2, the mycelial growth was good in the plots where the content of KNO_3 was between 50 and 80 mg, but the sporulation decreased with the quantity of potassium nitrate.

Table 2 Sporulation and mycelial growth of *Phytophthora infestans* on the media having different quantity of potassium nitrate

Content of KNO_3 mg/20cc	Days elapsed after inoculation 5	10	15	20	25	30	35	40	Spore formation	pH of medium
0	0.5	0.8	1.3	1.7	2.4	3.9	4.7	5.0	++++	5.6
10	0.5	0.8	1.1	2.4	3.0	3.9	4.7	5.2	++++	5.4
20	0.6	1.2	1.3	2.3	2.9	3.8	4.5	5.0	+++	5.6
30	0.5	1.0	1.2	1.5	1.7	2.4	3.0	3.6	+++	5.4
40	0.5	0.8	1.9	3.2	4.0	4.5	5.2	5.8	+++	4.8
50	0.5	0.8	1.5	3.5	4.1	5.4	6.1	6.4	++	4.8
60	0.5	0.9	1.5	2.8	3.6	4.7	5.8	6.4	++	6.0
70	0.6	0.8	1.2	3.4	4.1	5.4	6.4	6.7	++	5.6
80	0.6	1.1	2.0	3.0	4.2	4.8	5.8	6.7	+	5.6
90	0.5	0.8	1.2	2.0	3.8	4.4	5.4	6.6	+	5.6

* Diameter of mycelial mat (cm)

Generally speaking, the sporulation of the present fungus is rather poor on potato

decoction agar. Sakai[10] stated that the mycelial growth was good in the media containing $NaNO_3$ and KNO_3 as nitrogen source. And he[11] discussed some factors for the sporulation of sporangia. Sporulation is influenced by complicated combination of rare element and environment conditions, however, C-N ratio might be one of important factors for the sporangial formation.

2) Difference of sporulation on upper and lower leaves

The grade of susceptibilty of potatoes to *Phytophthora infestans* varies with the varieties and maturity of plants. The writers investigated the sporangial formation on potato leaves different in their maturity (upper and lower location). Varieties used were Irish Cobbler, Hokkaishiro, Kintoki-imo, Kamiya-imo No.1, and Benimaru. Tubers of these varieties were planted on the University Farm on April 15. From June 10 (initial stage of flowering) to the end of this month, the present investigations were carried out. In the first experiment, leaves were cut into pieces of 0.7 cm in diameter with cork borer containing spotted area and conidia on them were suspended in 0.5 cc of water, and the number of conidia in 5 microscopic fields was measured. Five drops were used per leaf, and investigations were repeated 20 times in each variety.

The results of these investigations were given in Table 3, and T test was carried out on the figures in this table. As shown in this table, the upper leaves have a tendency of producing conidia more abundantly than in the lower leaves in any of the variety used both in the field and in the laboratory. In Irish Cobbler, the spotted area in the upper leaves was larger than in the lower ones until 2 days after inoculation. Later, however, the lower leaves had larger spotted area than the upper ones.

Table 3 Number of conidia on upper and lower leaves

Variety	Experiment	Number of sporangia		Degree of freedom	t_0	t
		Upper leaf	Lower leaf			
Irish Cobbler	Field	467	277	24	5.09	2.80*
	Artificial inoculation	129	112	19	4.63	2.86*
Hokkai shiro	Field	222	218	19	3.88	2.86*
	Artificial inoculation	137	133	19	4.73	2.86*
Kintoki-imo	Field	302	127	19	4.53	2.86*
	Artificial inoculation	127	114	19	4.71	2.86*
Kamiya-imo No. 1	Field	125	58	19	4.53	2.86*
	Artificial inoculation	155	126	19	3.04	2.86*
Benimaru	Fjeld	194	127	19	12.11	2.86*
	Artificial inoculation	230	180	19	4.89	2.86*

* means significant at less than 1 % level

Yamamoto and Shimada[21] stated that there might have some correlation between the spotted area of potato late blight and total nitrogen content. They recognized the tendency that spotted area was larger in lower leaves than in upper leaves in every variety used, i. e. Benimaru, Kintoki-imo, Norin No.1 and Hokkaishiro. Accordingly it seems likely to have negative correlation between the sporulation and spotted area of late blight.

3) Contents of nitrogen and sugars on upper and lower potato leaves

As described above, abundant sporulation was given in the lower leaves than in upper leaves. For this reason, quantitative difference of some chemical components

and the enzymic activity might take part. To elucidate these points, analysis of total nitrogen, protein nitrogen, and non-protein, reducing sugars and non-reducing sugars was carried out.

Potato tubers (variety Irish Cobbler, Kennebec, Interspcific hybrid 48005-68, Norin No.1 and Kintoki-imo) were planted on April 20, and sampling for analysis was done on June 30th. Most of the upper leaves and the 3rd leaves from the lowest were dried at 90°C for one hour and were kept at 60°C for 24 hours. After drying, these leaves were pulverized. Total nitrogen was measured by Kjehldahl method, protein nitrogen was determined by Barnstein method. Non-protein nitrogen was represented by the difference of total and protein nitrogen. Quantity of reducing sugars was given as glucose by Bertrand's method and non-reducing sugars was shown as the difference of total and reducing sugars.

Yamamoto, Kimura and Kudo[19] stated that the quantity of total and protein nitrogen was less in lower leaves than in upper leaves in every variety used. Yamamoto and Shimada[21] also showed much higher total nitrogen content in Irish Cobbler, Warba, Kennebec, and Interspecific hybrid 48005-68.

Table 4 Quantity of nitrogen in upper and lower leaves in 5 different potato varieties

Variety	Location of leaf	Percentage of dried matter to fresh weight	Total nitrogen		Protein nitrogen		Non-protein nitrogen	
			mg per lg of dry matter	Percentage to fresh weight basis	mg per lg of dry matter	Percentage to fresh weight basis	mg per lg of dry matter	Percentage to fresh weight basis
Irish Cobbler	Upper	14.80	38.995	0.577	34.560	0.511	4.435	0.066
	Lower	11.50	36.785	0.423	32.871	0.378	3.914	0.045
Kennebec	Upper	15.33	62.695	0.961	61.320	0.940	1.375	0.021
	Lower	7.50	38.045	0.285	33.763	0.283	0.282	0.002
Intersp. 48005-68	Upper	16.12	44.365	0.715	43.000	0.593	1.365	0.112
	Lower	13.23	34.255	0.453	33.670	0.445	0.585	0.008
Norin No.1	Upper	16.00	40.630	0.730	37.834	0.605	2.796	0.125
	Lower	10.65	34.885	0.372	33.476	0.357	1.409	0.015
Kintoki-imo	Upper	17.00	34.255	0.583	33.037	0.562	1.208	0.021
	Lower	12.50	29.200	0.365	28.680	0.349	0.520	0.016

As shown in Table 4, the quantity of total and protein nitrogen was higher in the upper leaves than in the lower leaves in every variety used. In the upper leaves of comparatively resistant varieties, Kennebec, Norin No. 1, Interspecific hybrid 48005-68, and Kintoki-imo have larger quantity of nitrogen. However, in susceptible variety, Irish Cobbler, it showed the lowest value. Difference in the quantity of total nitrogen between the upper and lower ones is striking in resistant Kennebec and susceptible Irish Cobbler.

As to the total, reducing and non-reducing sugars, upper leaves have much more quantities than those lower leaves in all varieties tested, but these are no definite tendencies among the total, reducing and non-reducing sugars (Table 5). Suzuki, Sugaya and Hashimoto[15] stated that the quantity of reducing sugar was much larger in the upper part of the plant than in the lower one. Yamamoto and Shimada[21] described that higher quantities of total sugars and reducing sugars were found in the resistant varieties than in the susceptible varieties in both upper and lower leaves.

Table 5 Sugar content of upper and lower leaves in 5 potato varieties

Variety	Location of leaf	Percentage of dried matter to fresh weight	Total sugar		Reducing sugar		Non-reducing sugar	
			mg per lg of dry matter	Percent fresh weight basis	mg per lg of dry matter	Percent fresh weight basis	mg per lg of dry matter	Percent fresh weight basis
Irish Cobbler	Upper	14.80	185.24	2.741	38.23	0.565	147.01	2.176
	Lower	11.50	117.91	1.356	33.11	0.381	84.80	0.975
Kennebec	Upper	15.33	124.22	1.904	24.55	0.376	99.67	1.528
	Lower	7.50	103.13	0.773	23.46	0.176	79.67	0.598
Interspec. 48005-68	Upper	16.12	120.25	1.938	19.23	0.309	101.02	1.628
	Lower	13.28	99.01	1.309	16.04	0.212	82.97	1.098
Norin No.1	Upper	16.00	149.63	2.394	29.73	0.476	119.90	1.918
	Lower	10.65	121.41	1.293	28.21	0.300	93.23	0.993
Kintoki-imo	Upper	17.00	144.82	2.461	33.53	0.570	111.29	1.882
	Lower	12.50	103.51	1.294	30.93	0.387	72.58	0.907

4) Sporulation on potato leaf-juice

According to Sniezko[14] the sporulation of the present fungus is only found when the aerial mycelium grow abundantly and very few conidia are given in the mycelium in the medium. Sakai[10] also reported the same phenomenon. The writers carried out some experiments on the sporangial formation of *Phytophthora infestans* using juice macerated from potato leaves of different varieties or different maturity.

Tubers of Irish Cobbler, Warba, and Kamiya-imo No.1 were planted on September 10th and sampling was done from November to December. Fresh leaves were macerated by Waring blender and filtered through filter paper, and then filtered Chamberland filter and placed into agar solution before coagulation. Meanwhile, the same sample was sterilized by autoclave under 2 atmospheric pressure. The experiments were repeated twice and the results were shown in Table 6 and 7.

Table 6 Sporulation of *Phytophthora infestans* on juice macerated from potato leaves

Variety	Location of leaf	Heated		*	Non-heated		*
		Diameter of mycerial mat cm	Sporulation		Diameter of mycerial mat cm	Sporulation	
Irish Cobbler	Upper	5.0	++		3.2	+	
	Lower	3.7	+		3.2	+	
Warba	Upper	3.0	++		2.5	+	
	Lower	3.0	-		2.5	-	
Kamiya -imo	Upper	3.5	+		3.5	+	
	Lower	3.0	+		3.5	+	

* - means no sporulation, + means 10, ++ means 11—20, +++ means more than 21 conidia in one microscopic field under 80 magnification

Table 7 Sporulation of *Phytophthora infestans* on juice macerated from potato leaves

Variety	Heated		Non-heated	
	Diameter of mycelial mat	Sporulation	Diameter of mycelial mat	Sporulation
Irish Cobbler	5.2 cm	++	2.1 cm	+
Kennebec	6.3	+++	5.4	+++
Kintoki-imo	4.8	+++	2.3	++
Norin No. 1	3.6	+++	2.8	++
Menominee	6.1	+++	3.0	++

On juice agar of Kennebec and Norin No. 1, more abundant conidial formation or good mycelial growth was recognized than on that of susceptible variety, Irish Cobbler.

5) Influence of gibberellin and naphthalene acetic acid on the sporulation of *Phytophthora infestans*

Gibberellin is known as a substance to cause the acceleration of growth and flowering of plants, crop and trees, and naphthalene acetic acid is known as a synthetic plant hormone to cause the elongation of cells and stimulation of budding, and the concentrated solution stimulates the swelling of tissues, occurrence of roots and parthenocarpy. The writers made some experiments on these compounds on mycelial growth and sporulation of *Phytophthora infestans*.

Gibberellin and α-naphthalene acetic acid were put in various concentrations to potato decoction agar. Innoclum was cultured for 30 days at 20°C. After 25 days the number of sporangia prcduced on the medium was investigated under a microscope.

Table 8 Influence of Gibberellin and α-naphthalene acetate on the mycelial growth and sporulation of *Phytophthora infestans*

Concentration	Gibberellin	*	N A A	*
	Diameter of mycelial mat	Sporulation	Diameter of mycelial mat	Sporulation
ppm	cm		cm	
0.00	3.5	++++	3.5	++++
0.01	3.5	+++	3.5	++++
0.05	3.7	+++	3.2	++
0.10	3.1	++	2.1	+
0.25	2.2	+	1.5	+
0.50	1.3	+	1.0	-
1.00	0.3	-	0.3	-
10.00	0.3	-	0.3	-
25.00	2.1	++	2.0	+
50.00	3.3	++	2.9	++
75.00	0.3	-	0.3	-
100.00	0.3	-	0.3	-

* + means 1~5, ++ means 6~10, +++ means more than 15 conidia in one microscopic field under 80 magnification

Both Gibberellin and α-naphthalene acetic acid in the concentration of 0.01 p p m showed almost the same level of growth or sporulation, but the higher concentration the lesser growth or sporulation was observed. In the concentration of 25-50 p p m *Phytophthora infestans* might be given slight stimulation.

In general, gibberellin is used in the concentration of 1-100 p p m in applying to plant bodies. In the concentration of more than 75 p p m, the mycelial growth and the sporangial formation was again checked.

About the carbon sources of *Phytophthora infestans*, Yamamoto and Kobayashi[20] and Sakai[10,13] have already reported. Yamamoto and Kobayashi[20] described that glucose, galactose gave the good mycelial growth but other carbon sources especially organic acids gave worse results. Sakai[10,13] reported the same tendency.

The writers' experiments on the glucose content to the mycelial growth and sporulation showed that the larger mycelial growth caused the abundant formation of sporangia in general, and maximum point was obtained at 40-50 mg plot in 20cc of medium.

Mix[9] stated that the nitrogen source influenced the formation of pycnidia and pycnospores of *Phyllosticta solitaris* and Sakai[10] recognized this point in *Phytophthora infestans* too. According to him, the lesser the nitrogen content was, the more abundant the sporangial formation was found in the experiment using potassium nitrate as a nitrogen source. Westergard and Mitchell[17] described the influence of C-N ratio and others to the perithecial formation of *Neurospora crassa*. In the case of *Phytophthora infestans*, the higher the C-N ratio is, the more abundant sporangial formation is caused until definite value. Of course, although the sporulation is influenced by various complicated environmental conditions and factors, e. g., C-N ratio might take part as one of the important factors.

In the previous experiments, the writers observed that the number of sporangia formed on upper leaves was much more than on lower leaves. From these observations the upper leaves might have more suitable conditions to the sporangial formation. The writers measured the nitrogen and sugar content in fresh potato plant and the results coincided with those of Yamamoto, Kimura and Kudo[19], Yamamoto and Shimada[28] stating that the upper leaves have much higher values than lower ones. Culture experiment, however, showed that the greater the quantity of potassium nitrate was, the lesser the sporangial formation was found. The result seems to show some contradiction to the writers' previous culture experiment in which they found out, the lesser the potassium nitrate was, the better was the sporangial formation. However, the condition for spore formation *in vivo* might be more complicated than in the experiment *in vitro*. Further studies will be required in these points.

Fenstel-Schönbrunn[4] stated that newly developed leaves have some substances which stimulate the growth of *Phytophthora infestans*. The writers observed the sporangial formation and myclial growth on agar plates including juice macerated from potato leaves (upper and lower), but no remarkable difference was observed. As to the juice macerated from potato tubers, better sporangial formation and mycelial growth was obtained in Kennebec and Norin No.1 which were more resistant than Irish Cobbler.

Gibberellin and α-naphthalene acetic acid inhibit the growth of *Phytophthora infestans* in the concentration of 0.04-0.05 p p m. As to the physiological significance of these substances more detailed studies are required.

3. Sporangial germination of *Phytophthora infestans*

It is widely known that sporangia of *Phytophthora infestans* have two types of germination, i.e., at the lower temperature (11-13°C) sporangia germinate indirectly liberating swarmspores, and at the higher temperatures (20-23°C) germinate directly by putting out germ-tubes.

To account for the mechanism of these two types of germination, the difference of osmotic pressure of sporangia, the respiration, the change of glycogen content by germination which is thought to be one of the source of energy, and phosphatase activity which might play an important role to the phosphorous metabolism should be taken into consideration.

1) Time required for the indirect germination of sporangia of *Phytophthora infestans*

H_1 (Race O) was used as test fungus. Potato tubers (Irish Cobbler) were cut in slices in the thickness of about 0.5 cm and the fungus cultured for 20 days was inoculated. These were put in moist chamber for 10 days at 21-22°C. Sporangia produced on these tubers were taken on microscopic slides with glucose solution in various concentrations and the total number of sporangia and the number of sporangia germinated were investigated under a microscope at 12°C.

Table 9 Time required for the liberation of swarmspores from sporangia of *Phytophthora infestans* at 12°C

Concentration of glucose solution (Mol)	Item	Time (Minute) 60	120	180
0.78	Number of conidia investigated	485	243	200
	Number of germinated conidia	190	200	180
	Percentage of germination	39.17	82.30	90.00
0.74	Number of conidia investigated	450	317	200
	Number of germinated conidia	113	227	160
	Percentage of germination	25.11	71.60	80.00
0.62	Number of conidia investigated	467	244	135
	Number of germinated conidia	24	180	95
	Percentage of germination	5.14	74.00	70.34
0.40	Number of conidia investigated	419	414	294
	Number of germinated conidia	20	130	197
	Percentage of germination	4.77	31.40	67.00

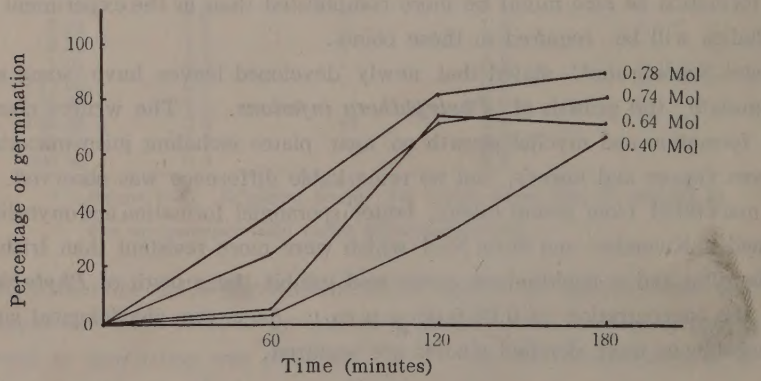

Fig. 1 Time required for the liberation of swarmspores from sporangia of *Phytophthora infestans*

Katsura[8] described that indirect germination of *Phytophthora capsici* showed 1.4 per cent after 15 minutes at 20-21°C, but likewise was completed the indirect germination in about 1 hour. Bonde described that *Phytophthora infestans* germinates indirectly for 6 days at 13-15°C. Cicarone[2] stated that 40-50 percent of indirect germination was at 18 °C and 5 per cent less was at 24°C in 1.5 to 2 hours.

According to Table 9, 70-80 % of the swarmspores liberate from the sporangia after 2 hours at 12°C. Cicarone[2] mentioned 1.5-2 hours, and Carlton[1] noticed 4 hours. Hidema and Kole[6] stated that the germ tubes were formed after 1.5 hours. Accordingly, every change of constituents of sporangia should be investigated within 3-4 hours.

2) Difference of osmotic pressure in the case of direct and indirect germination of the sporangia of *Phytophthora infestans*

The outer conditions which influence the germination of sporangia i.e., temperatures, humidity, light and inorganic or organic elements as well as the inner conditions the change of contained constituents and enzymatic activity might be considered. And the osmotic pressure might also be influenced by the environmental condition in the case of sporangial germination.

Osmotic pressure was measured after plasmolysing the sporangia in different concentration of glucose. Direct germination was measured in 0.3cc of glucose solution in different concentrations in moist chamber at 20°C. Indirect sporangial germination was measured after 2 hours at 11°C.

Table 10 Osmotic pressure of sporangia of *Phytophthora infestans* in the case of germination

		Direct germination			Indirect germination		
Concentration of glucose (M)	Osmotic pressure (Atm) of sporangia	Number of sporangia measured	Plasmolysed sporangia	Percentage of plasmolysis	Number of sporangia measured	Plasmolysed sporangia	Percentage of plasmolysis
0.40	9.45	926	9	0.97	—	—	—
0.45	10.63	463	44	9.50	—	—	—
0.50	11.81	680	87	12.79	—	—	—
0.52	12.28	496	55	11.31	135	0	0
0.56	13.22	350	46	13.14	415	0	0
0.58	13.69	530	81	15.47	293	0	0
0.60	14.17	156	42	26.92	365	0	0
0.62	14.64	580	181	31.20	842	6	0.71
0.64	15.01	372	110	29.56	531	8	1.41
0.68	16.05	210	61	29.04	150	16	17.33
0.70	16.53	522	210	40.22	172	51	29.65
0.74	17.48	211	85	40.28	123	43	34.95
0.78	18.42	341	185	54.25	233	219	93.99

As shown in Table 10 and Fig. 2, osmotic pressure of sporangia in the case of indirect germination showed its value in higher level than in direct germination. Plasmolysis of sporangia in direct germination begins at 0.42 molar concentration and increases gradually and 54.25 per cent of plasmolysis was given at 0.78 mol. However in the case of direct germination, the remarkable increases are recognized from 0.62 ml and 93.99 per cent of plasmolysis was given at the concentration of 0.78 mol.

3) Respiration of sporangial suspension of *Phytophthora infestans* during germination.

The osmotic pressure of sporangial cells in direct germination was higher than in indirect germination. And it might be supposed that some rapid change of inner constituents of spores might take place in the case of indirect germination more than of direct germination. To account for this point, the writers measured the respiration of sporangia in germination.

Late blight lesion were rinsed in distilled water several times and diseased leaves were kept in moist chamber at 20-23°C. After 24 hours, abundant sporangia were produced on both sides of the leaves. Sometimes sporangia on tubers were also used. The measurement of respiration was given by the use of Warburg's manometer at 12 and 22°C for 30-200 minutes. 5 cc of sporangial suspension and buffer solution were put into main conpartment of vessel, 0.5 cc of 10 per cent KOH and a bit of filter

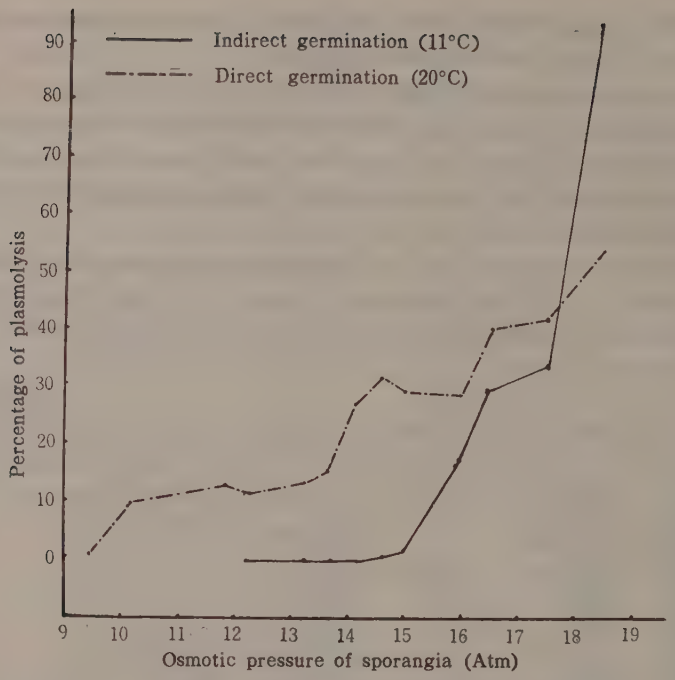

Fig. 2 Difference of osmotic pressure of sporangia of *Phytophthora infestans* in case of direct and indirect germination

paper were put into side bulb of the vessel of Warburg's manometer. Sörensen's phosphate solution was used as buffer solution. The concentration of sporangial suspension was 40-60 conidia in one microscopic field under 80 magnification.

The change of respiration was measured using glucose and Na-adenosinetriphosphate solution as well as distilled water.

Respiration of sporangia in the case of indirect germination raised more suddenly after 10 minutes than in it of the direct germination and it decreased suddenly after 2 hours.

Table 11 Sporangial respiration of *Phytohhthora infestans* 0_2 uptake (ml)

Direct germination (at 22°C)				Indirect germination (12°C)		
Time (min)	Distilled water	10%glucose solution	Na-ATP 10^{-4}Mol solution	Time (min)	Distilled water	10%glucose solution
0	0.00	0.0	0.00	0	0.00	0.00
30	0.85	3.6	0.81	30	6.96	21.60
60	0.00	5.4	5.67	60	9.57	24.10
90	0.85	7.2	7.29	90	12.18	27.90
120	1.70	10.8	8.91	150	10.79	10.44
200	3.40	16.2	9.71	200	—	—

N. B. Measurement was done by 5 cc of sporangial solution and buffer solution

After 2 hours sudden decrease of respiration was observed, and it was presume that swarmspores might be produced in this stage. Respiration increases with the progress of time in direct germination at 22°C. Slight raising of respiration was recognized in Na-ATP 10^{-4} molar solution than was recognized in distilled water.

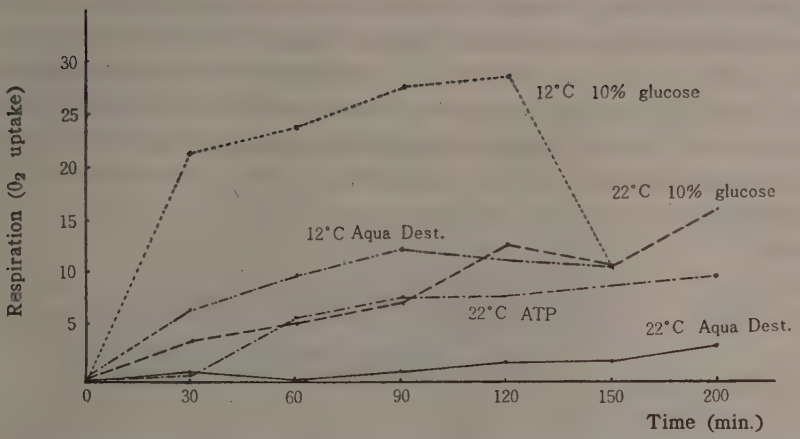

Fig. 3 Respiration of sporangial suspension of *Phytophthora infestans* in germination

4) Change of glycogen in sporangial germination of *Phytophthora infestans*

Sporangia absorb water and swell as an initial step in germination. To acquire the energy necessary for sporangial germination, glycogen and other reserve materials should be decomposed in lower compounds, and the molar concentration of substances within protoplasm should be raised and the respiration must be enhanced. From such way of thinking, the change of the quantity of glycogen was taken into consideration.

To detect the glycogen in protoplasm within the sporangia, Baul Feulgen stain method and Best-carmine stain method after Bensley [5,7] were used.

Baur Feulgen stain method One gram of basic fuchsin was put into 100 cc of distilled water and solved by heating, and 20 cc of 1 N hydrochloric acid and one gram of sodium bisulfite was added. Sporangial suspension was taken into a centrifuge tube and 4 per cent of chromic acid was put for 30 minutes. Then it was treated with Feulgen reagent for 10-15 minutes. After centrifugation, sporangia were rinsed with distilled water twice and were rinsed with diluted sulphuric acid one time, and then rinsed with distilled water. Under a microscope, glycogen was recognized within protoplasm as a dark purple color and the intensity of color was graded by the quantity of glycogen.

Best carmine stain method 2 grams of carmine, 1 gram of potassium chloride were solved in 60 cc of distilled water, then 20 cc of concentrated ammonium was added and after 24 hours it was used as carmine stain stock solution. Immediately before the use, 15 cc of conidial suspension and 30 cc of methanol were added to 10 cc of stock solution. 5 cc of conidial suspension (40-50 sporangia in one microscopic field under 80 magnification) was taken in centrifuge tube and treated with Fresh carmine stain for 20 minutes. After centrifugation, sporangia were rinsed with methanol twice and the same procedure was repeated. Glycogen was recognized as brilliant red color in protoplasm. The color was graded from 0.5 to 2.5. The experiments were repeated threee times.

The results of staining showed that glycogen in sporangia increased in direct germination and the increase continued for 3 hours, meanwhile in the case of indirect germination, sudden decrease of glycogen was recognized just before the liberation of swarmspores. Some difference was obserevd by Best carmine method and Baur Feulgen method, i.e., at 12°C, in Baur Feulgen methcd the increase was observed at 3 hours but in Best carmine method the increase was observed at 3 hours and thereafter no increase was recognized.

Table 12 Change of glycogen in sporangia at the time of direct and indirect germination

Time (hours)	Staining method	Indirect germination (12°C)	Direct germination (23°C)
0	Best Carmine	1.00	1.00
	Baur-Feulgen	0.25	0.50
1	Best Carmine	1.35	0.85
	Baur-Feulgen	1.14	1.14
2	Best Carmine	1.71	1.75
	Baur-Feulgen	1.00	1.28
3	Best Carmine	2.00	2.14
	Baur-Feulgen	0.28	1.28
4	Best Carmine	2.00	3.00
	Baur-Feulgen	0.15	1.58

N. B. Figures were given in relative value shown as 1.00 at the initial stage of germination

5) Change of acid phosphatase during the germination of direct and indirect sporangial germination.

Concerning the glycogenesis, activation of some enzymes might be connected, so the change of acid phosphatase activity during germination was investigated.

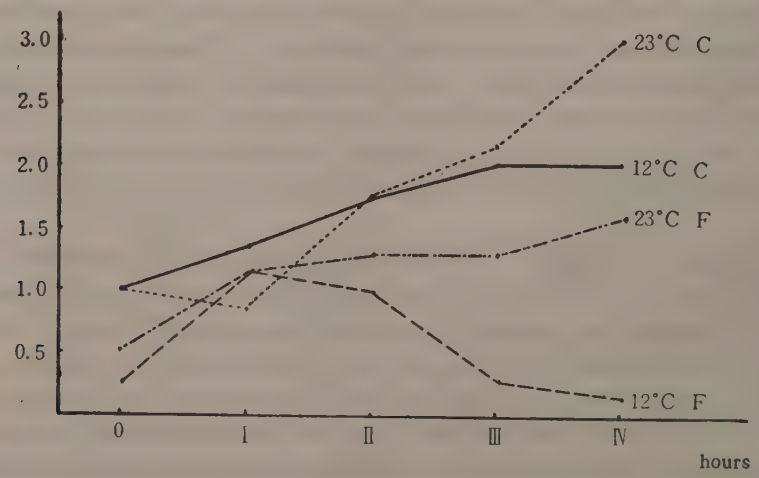

Fig. 4 Change of glycogen in sporangia at time of direct and indirect germination

C : Best Carmine stain method

F : Baur Feulgen stain method

As a substrate 3.2 per cent solution of sodium glycerophosphate was used. 4cc of 0.1 molar concentration of acetic buffer solution was added to it to make pH 5.1 and total volume was made up to 6 cc with the addition of 0.1 molar lead nitrate and 0.6 cc of distilled water. Besides them, 2 per cent of acetic acid solution and 1 per cent of ammonium sulfide solution were prepared. To 5 cc of fresh sporangial suspension, substrate solution was kept at 37°C for 30 miuutes. This was rinsed with distilled water twice. After centrifugation, it was treated with ammonium sulfide solution for 2-3 minutes and after rinsing with distilled water again, preparation was observed under microscope. Acid phosphatase was recognized as brown or black precipitate within the protoplasm of sporangia. The color was given in 5 grades, and the figures were shown as an average of three replication.

Acid phosphatase activity during direct and indirect germination were shown in Table 13 and Fig. 5. The activity was slightly higher during indirect germination than in indirect germination. It was, however, very difficult to find some relation of the activation of acid phosphatase during germination.

Table 13 Activity of acid phosphatase during direct and indirect germination

Time (hours)	Indirect germination (12°C)	Direct germination (23°C)
0	1.20	1.10
1	1.61	1.33
3	1.55	1.18
4	1.22	1.00

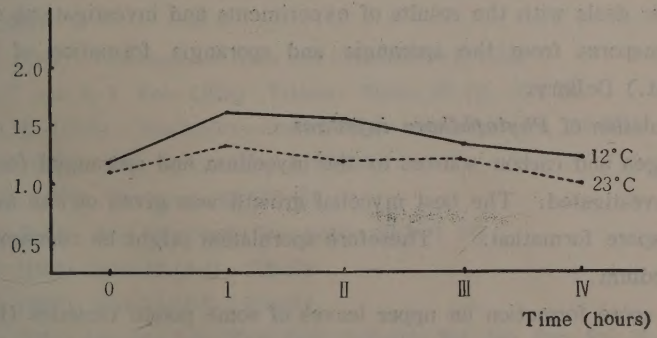

Fig. 5 Activity of acid phosphatase in sprangia during direct and indirect germination

4. Discussion on sporangial germination of *Phytophthora infestans*

According to Cicarone[2], indirect germination of *Phytophthora infestans* begins during 2-2.5 hours and finishes after 3-4 hours. In the writers' experiments, 70-80 percent of swarmspores liberates from sporangia at 12°C after 2 hours. Osmotic pressure of sporangia in indirect germination showed higher value than that of in direct germination. This might suggest the possibility of physiological difference of porangia in these cases and it might be connected with the respiration of them.

Respiration of sporangia in the case of indirect germination raised 10 minutes later and decreased suddenly after 2 hours comparing with that of indirect germination. It seem possible that the increase of osmotic pressure by the sudden change of inner constituents within the sporangia might lead to the abrupt increase of respiration. In the direct germination, the respiration increases gradually with the progress of time too.

Yamamoto[18] stated the liberation of swarmspores of *Phytophthora infestans* was stimulated with the addition of ATP and was inhibited with the DNP and therefore oxidative phosphorylation might be concerned with the germination and the neccessity of energy should be regarded. The writers observed the raising of respiration by the addition of 10^{-4} molar concentration of Na-ATP comparing with that of distilled water. This seems some correlation might exist between respiration and germination.

Glycogen in sporangial cells increases gradually in the case of direct sporangial germination of *Phytophthora infestans* and it continues, even later in the case of indirect germination after 2 hours later, namely, after the formation of swarmspores, the decrease was recognized. It seems possible that glycogen might take some change within sporangia.

Acid phosphatase showed slightly higher activity in indirect germination than in direct germinaton. Unless alkaline phosphatase and other enzyme activity were kept in mind, the concept of glycogenesis within the sporangia could not be understocd.

Summary

This paper deals with the results of experiments and investigations on the germination of swarmspores from the sporangia and sporangia formation of *Phytophthora infestans* (Mont.) DeBary.

1) Sporulation of *Phytophthora infestans*

i) Nitrogen and carbon sources to the mycelium and sporangial formation of the fungus were investigated. The best mycelial growth was given on the medium which is suitable for spore formation. Therefore sporulation might be concerned with C-N ratio of the medium.

ii) The spore formation on upper leaves of some potato varieties (Irish Cobbler, Hokkai-shiro, Kintoki-imo, Benimaru) were much better than on lower leaves.

iii) The writers measured nitrogen and carbon contents in upper and lower leaves of potato plants. The upper leaves contained more nitrogen and glucose than the lower leaves, however, positive correlation between the spore formation and the contents of the protein nitrogen or reducing sugar in leaves was not proved.

iv) The significant difference of spore formation cultured on potato leaf dextrose agar from these potato varieties, and also from upper or lower leaves was not observed. The mycelial growth and spore formation were much better on the potato dextrose agar of resistant varieties than that of susceptible varieties.

v) The mycelial development and sporulation of the fungus were checked in

nutrient solution containing higher concentration of gibberellin and NAA than 0.1 ppm.

2) Germination of swarmspores of *Phytophthora infestans*

i) The swarmspores were liberated from sporangia at the percentage of 70–80 at 12°C for 2 hours.

ii) The osmotic pressure of sporangia in the case of indirect germination was higher than in direct germination.

iii) The respiration of spores suspended in water increased gradually when the fungus germinated directly, but in the case of indirect germination, it increased rapidly and dropped down after 2 hours.

iv) The content of glycogen within spores increased gradually when the spore germination takes place directly, but in the case of indirect germination it decreased after 2 hours.

v) The significant difference of acid phosphatase activity was not observed in either direct or indirect germination.

Literature cited

1. Carton,F.,J.John., D.O.Smot, R.A.Quin, R.A.Rhode and E.E.Elliot (1955) Phytopath. 45 (12): 673–676
2. Cicarone, A. (1949) Riv. Agric. Subtrop. 43: 1-3, 22-28, (Ref. in Rev. Appl. Myc. 30 (2): 68-69, 1951)
3. Enomoto, S. (1952) Mem. Hokkaido Univ. Jap. Ed. 1(3) : 256-274
4. Fenstel-Schönbrunn, R. (1956) Zbl. Bakt. Abt. 2, 108: 19–20, 513–53, (Ref. in Rev. Appl. Myc. 35 :220, 1956)
5. Glick, D. (1949) Techniques of histo and cytochemistry, 1–531
6. Hidema, T. and A. F. Kole (1954) Tijdschr. Plzeki. 60 (3) : 138–139
7. Ichikawa, K. (1953) Cytochmistry and its theory and techniques, 379–382
8. Katura, K. (1956) Bull. Saikyo Univ. 8: 97–106
9. Mix, A. T. (1933) Phytopath. 23: 503–524
10. Sakai, R. (1955) Ann. Phytopath. Soc. Japan 19(3–4): 178
11. Sakai, R. (1955) Ibid. 19 (3–4) : 178–179
12. Sakai, R. (1957) Ibid. 22 (4–5) : 211–214
13. Sakai, R. (1955) Ann. Rep. Lab. Plant Path. Hokkaido Nat. Agr. Exp. Sta. 28 (mimeographed)
14. Sniezko, S. F., J. B. Carpenter, E. P. Lowe and G. J. Jacob (1947) Phytopath. 37: 635–649
15. Suzuki, H., K. Sugaya and Y. Hashimoto (1951) Ann. Phytopath. Soc. Japan 15: 88–89
16. Togashi, K. (1949) Biological characters of plant pathogens temperature relation, 214–219
17. Westergard, M. and H. K. Mitchel (1947) Amer. Journ. Bot. 34 : 573–577
18. Yamamoto, M. (1957) Bull. Shimane Agr. Coll. 5 : 24–29
19. Yamamoto, M., M. Kimura and K. Kudo (1951) Ann. Rep. Lab. Plant Path. Morioka Exp. Farm, Tohoku Nat. Agr. Exp. Sta., 32–37
20. Yamamoto, M., and Y. Kobayashi (1951) North Eastern Agriculture 7(1): 23–25
21. Yamamoto, M., and K. Shimada (1958) Bull. Shimane Agr. Coll. 6(A): 91–99

馬鈴薯疫病菌分生胞子の形成ならびに発芽に関する生理学的研究

山本昌木・谷野淳一

摘　　要

1. 馬鈴薯疫病菌の胞子形成

i) 馬鈴薯疫病菌の菌糸発育に及ぼす炭素源および窒素源の影響と胞子形成の関係を調べたところ，菌叢の発育が良好なところで胞子形成は多い傾向がみられ，C-N率が 胞子形成に関係するらしい．

ii) 馬鈴薯上下葉における胞子形成は，男爵薯，北海白，金時薯，神谷1号，紅丸共に上葉において 下葉より多い傾向がみられた．

iii) 馬鈴薯上下葉における窒素含量および糖類含量を測定したところ，全窒素，全糖類共に 下葉より上葉において含量が多く、蛋白態窒素および還元糖と胞子形成量との間には、一定の関係は認められなかつた．

iv) 馬鈴薯茎葉搾汁寒天培地上で胞子形成を観察したところ、茎葉においては、男爵薯、ワーバー、神谷1号の3品種間に，上下葉の区別なしに差はみられなかつた。塊茎搾汁液については罹病性品種の男爵薯より抵抗性品種のケネベツク，農林1号などにおいて菌糸発育量および胞子形成が多いのが注目された．

v) ジベレリンおよびα-ナフタリン醋酸の 0.1ppm 以上の濃度では，菌糸発育ならびに胞子形成は共に抑制される傾向があつた．

2. 馬鈴薯疫病菌分生胞子の発芽

i) 分生胞子は12°Cで 2時間経過すると70−80%が游走子を逸出した．

ii) 直接発芽時よりも間接発芽時における分生胞子の浸透圧は高かつた．

iii) 馬鈴薯疫病菌胞子懸濁液の発芽時における呼吸量は直接発芽においては 徐々に高まつていくが，間接発芽においては急激に高まり，2時間経過すると低下してきた．

iv) 直接発芽時においてグリコーゲンは 徐々に増加してくるが，間接発芽時においては2時間経過後より減少してきた．

v) 解糖酵素作用として酸性フオスフアターゼを測定したが，直接および間接発芽に おいてあまり差は認められない